

S6.P11

Dehydro-flavonolignans from silymarin are potent inhibitors of lipid peroxidation in isolated respiring rat heart and liver mitochondriaMartin Modrianský^a, Aleš Dvořák^b, David Biedermann^c, Vladimír Křen^c, Petr Ježek^b, Martin Jabůrek^b^aDepartment of Medical Chemistry and Biochemistry, Palacký University, Czech Republic^bDepartment of Membrane Transport Biophysics, Institute of Physiology, ASCR, Czech Republic^cInstitute of Microbiology, Czech Republic

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Flavonoids and polyphenolic compounds are known for their cytoprotective and antioxidant properties, but the interactions of these compounds with isolated respiring mitochondria are largely unexplored. Here, we studied selected active constituents of silymarin, flavonolignan complex from the seeds of *Silybum marianum*, which is known for its cytoprotective properties [1]. We used the major silymarin compound silybin, as well as several minor components, such as silydianin, silychristin, 2,3-dehydrosilybin, 2,3-dehydrosilydianin and 2,3-dehydrosilychristin, and tested their effects on respiration, membrane potential and the kinetics of mitochondrial lipid peroxidation, using isolated respiring rat heart and liver mitochondria. Compared to the dehydrosilybin, which causes a partial dissipation of the mitochondrial protonmotive force [2], all other compounds had a negligible effect on mitochondrial respiration and membrane potential in the concentration range tested. All compounds, however, were potent inhibitors of spontaneous mitochondrial lipid peroxidation, although with different potencies. Dehydrosilychristin, dehydrosilybin and dehydrosilydianin were the most potent inhibitors, with IC₅₀ values below 0.5 μM (2.5 nmol per mg of protein). These results contribute to our understanding of the interactions of natural polyphenolic compounds with mitochondria and suggest that not the major flavonolignan silybin, but the minor components are responsible for the antioxidant properties of the silymarin mixture. These results further indicate that flavonolignans may execute their antioxidant and cytoprotective actions through inhibition of lipid peroxidation originating from respiring mitochondria. This work was supported by the Czech Science Foundation (GACR 301/11/0662).

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S6.P12

Low rate of ROS production and elevated levels of oxidative stress markers in the extraordinarily long-lived bivalve mollusc *Arctica islandica* – A conundrum for the oxidative stress theory of agingDaniel Munro^a, Jason Treberg^b, Nicolas Pichaud^a, Pierre U. Blier^a^aUniversité du Québec à Rimouski, Canada^bUniversity of Manitoba, Canada

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The finding of an inverse correlation between ROS production by isolated mitochondria and species longevity would strongly support

the mitochondrial oxidative stress theory of aging. After a first decade of research (ten studies on mammals, birds and insects) providing evidence for such relation, the last decade (nine studies) provided divided results. Reanalysis correcting for unequal phylogenetic distances and body sizes further provided controversial results [1]. The emerging biogerontology model of extremely long-lived bivalve molluscs provided us with an opportunity for a test of this relationship while escaping the need to statistically correct for unequal body mass and phylogenetic distances. Measurements of hydrogen peroxide efflux revealed lower rates of ROS production by isolated mitochondria from the longest-lived metazoan, the bivalve *Arctica islandica* (maximum reported longevity = 507 years) when compared with two taxonomically related short-lived species of similar size. The differences prevail during forward, reverse, and convergent electron flow. Compared to the shortest-lived species (*M. arenaria*), *A. islandica* did not present higher maximal activity of the alternative oxidase (AOX). Reduced ROS production rates may rather be partly explained by lower Complex I and II maximal catalytic capacity per unit mitochondria maintain a lower degree of electronic reduction of complex I and III oxygen reduction sites for similar maximal state III oxygen consumption. Contrary to our expectations, whole tissue lipid oxidation assays, thiobarbaturic acid reactive substances and lipid hydroperoxides levels increased with increasing longevity among four species including *A. islandica*. Whereas our study supports an inverse correlation between longevity and the rate of ROS production by mitochondria of bivalves, it also suggests that an elevated oxidative injury may not be incompatible with extreme longevity, the latter being also observed in the long-lived rodent (*Heterocephalus glaber*).

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S6.P13

Probing the interaction between the N-terminal domain of *Escherichia coli* AhpF and AhpC

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Oxidative stressors are employed by host systems to defend it from pathogenic bacteria. Consequently, several pathogens have developed sophisticated mechanism to evade such insult from the host. To do this, pathogens depend on enzymes like catalases and the alkylhydroperoxide reductase (AhpR) system to convert harmful H₂O₂ and organic peroxides to water and alcohol, respectively. The AhpR consists of AhpC which directly reduces H₂O₂ and organic peroxides and AhpF, which is an NADH-linked flavoprotein, a reductase of the AhpC. Together, this system contributes to pathogenesis (1) and resistance (2) in several pathogens. Despite the potential of targeting this system with drugs, the exact interaction, and therefore the binding interface between the AhpC and AhpF has not been characterized yet. In this study, with knowledge that the N-terminal domain (NTD) of the AhpF is responsible for binding AhpC, the NTD of the *E. coli* AhpF has been purified to high homogeneity and used for small angle X-ray scattering (SAXS) experiments. The SAXS-data indicate a highly

stable protein and confirm the solution state of the construct. Using Isothermal Titration Calorimetry (ITC) we have examined the interactions involved in the catalytic cycle of the enzyme. The interaction is endothermic and the K_d was calculated to be 3.2 μ M. Furthermore, with Nuclear Magnetic Resonance (NMR) spectroscopy, we are attempting to zero in on the exact residues and binding interface of the interaction. Information from this study will facilitate drug development against known pathogens depending on AhpF.

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S6.P14

Pre-diabetes disrupts testicular PGC1- α /SIRT3 axis prompting oxidative stress

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Pre-diabetes, a risk factor for type 2 diabetes development, alters testicular physiology disturbing in particular its metabolism and bioenergetic capacity, which favors oxidative stress. Oxidative stress occurs due to uncontrolled reactive oxygen species (ROS) production and an inefficient ROS scavenging system. Peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α) and Sirtuin 3 (SIRT3) act synergistically as key inducers of ROS-detoxifying enzymes. In this regard, we hypothesized that pre-diabetes disrupts testicular PGC-1 α /SIRT3 axis enhancing an oxidative environment and contributing to the decline of reproductive performance. Using a high-energy-diet (HED) induced pre-diabetic rat model, we evaluated testicular levels of PGC-1 α and its downstream targets, nuclear respiratory factor 1 (NRF-1) and 2 (NRF-2), mitochondrial transcription factor A (TFAM) and SIRT3. We also assessed oxidative stress parameters, such as antioxidant capacity, lipid peroxidation and protein carbonylation. Protein levels were quantified by Western Blot. Antioxidant capacity and lipid peroxidation were evaluated spectrophotometrically. Protein carbonylation was assessed by immunoblot. After HED treatment animals displayed the characteristics of a pre-diabetic state, such as slightly increased blood glucose levels and impaired glucose tolerance. Both testicular PGC-1 α and SIRT3 levels were significantly decreased. NRF-1, NRF-2 and TFAM were not altered. Concerning oxidative status parameters, pre-diabetes decreased testicular antioxidant capacity and increased both lipid and protein oxidation. In sum, HED-induced pre-diabetes down-regulates testicular PGC-1 α /SIRT3 axis, favoring oxidative damage. As the synergistic role of PGC-1 α and SIRT3 proteins is blunted in

pre-diabetic animals, an entire oxidative cascade is triggered with associated increase of testicular lipid and protein oxidative injuries and decreased testicular antioxidant capacity, which occur earlier in the development of type 2 diabetes and may later have a severe negative impact in male fertility.

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S6.P15

Influence of the Ca²⁺-independent phospholipase A2 (iPLA2) on the electron-transport-chain dependent ROS generation in brain mitochondria

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Oxidative stress is a factor for the pathogenesis of many diseases, like neurodegeneration in Alzheimer's disease. Therefore, drug-based prevention of reactive oxygen species (ROS) generation is important. The Ca²⁺-independent phospholipase A2 (iPLA2), which liberates free fatty acids (FFA) by hydrolyzing the sn-2 ester bond of membrane glycerophospholipids, has been found in various mammalian mitochondria. According to a classical concept, the activity of the inner mitochondrial membrane-associated iPLA2 removes oxidatively damaged fatty acids for lipid remodeling and repair. Since mitochondria are main ROS producers in cells, the question arises, whether the iPLA2 has antioxidative defense, to attenuate the formation of electron transport chain (ETC)-associated superoxide (O₂⁻), and thereby oxidative stress, by mild-uncoupling. We examined the influence of iPLA2 on ETC-associated ROS generation in rat brain mitochondria (RBM). First, docosahexanoic acid (DHA), a major reaction product of iPLA2, was used to adjust mild-uncoupling in succinate-oxidizing RBM and, to impair reversed electron transport (RET) in ETC. Low DHA concentrations diminish RET-dependent ROS generation by mild-uncoupling. This was mostly due to the adenine nucleotide translocase. In contrast, when mitochondria oxidize glutamate plus malate and, thereby support the forward electron transport (FET), DHA enhanced mitochondrial ROS generation. In addition, to reduce the endogenous mitochondrial pool of FFA, mitochondria were treated with the iPLA2-inhibitor bromoenol lactone (BEL). BEL-treated and succinate-oxidizing mitochondria show enhanced ROS generation, but are slightly depolarized in comparison to the untreated control. This finding contradicts the view that iPLA2 inhibition abolishes mild uncoupling and, consequently, enhances the mitochondrial membrane potential. On the contrary, we explain the increase of the ROS release by BEL-treated RBM with a diminished content of reduced glutathione. Thus, we disprove the view that iPLA2 attenuates oxidative stress in brain mitochondria. Further work should elucidate mechanisms of pathogenesis of neurodegenerative disorders based on the dysregulated iPLA2 [1].

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